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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61L 25/00

A1

(11) International Publication Number: WO 97/29792

(43) International Publication Date: 21 August 1997 (21.08.97)

(21) International Application Number: PCT/US97/02614

(22) International Filing Date: 19 February 1997 (19.02.97)

(30) Priority Data:
60/011,973 20 February 1996 (20.02.96) US
Not furnished 18 February 1997 (18.02.97) US

(60) Parent Application or Grant
(63) Related by Continuation
US Not furnished (CON)
Filed on 18 February 1997 (18.02.97)

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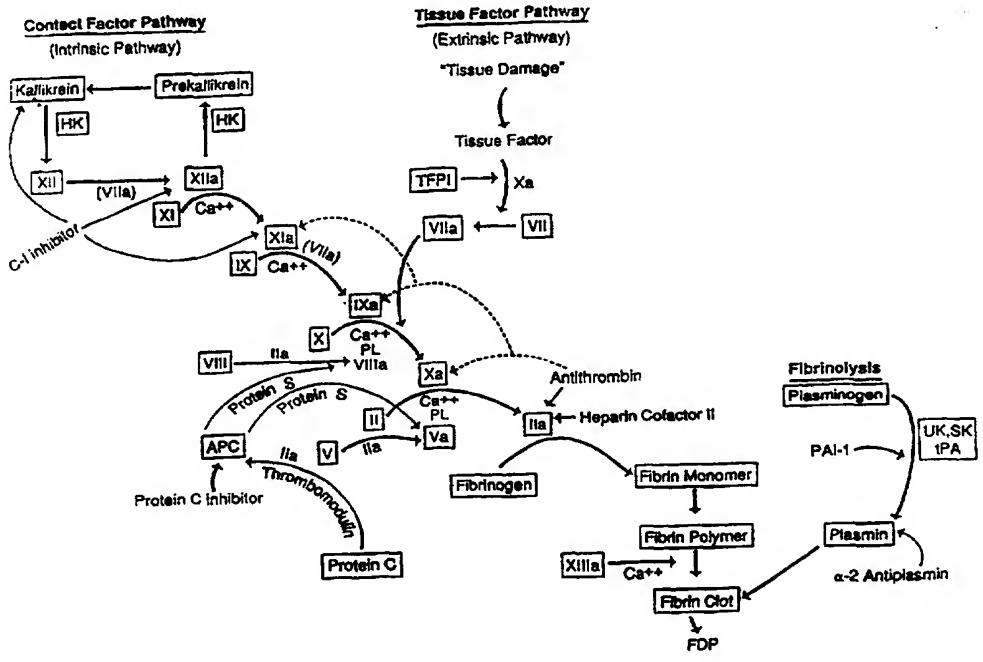
(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

**Published**
*With international search report.*
*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: TISSUE SEALANT COMPOSITIONS AND METHODS OF USE THEREOF




(57) Abstract

Novel tissue sealant compositions are disclosed herein which can be formulated as a single-component. The compositions contain as essential elements thromboplastin and fibrinogen. Additional blood clotting factors such as Factors II, V, VII, X, and XIII can also be added. Also provided are methods for promoting tissue adhesion and/or hemostatus by administering the tissue adhesive compositions described herein.

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# TISSUE SEALANT COMPOSITIONS AND METHODS OF USE THEREOF

This application claims priority from provisional application No. 60/011,973, filed February 20, 1997, incorporated herein in its entirety.

## TECHNICAL FIELD OF THE INVENTION

This invention relates to fibrin based tissue sealant compositions and more specifically, to thromboplastin-containing tissue sealant compositions which are capable of effectuating formation of a fibrin based clot *in situ*..

## BACKGROUND ART

Blood coagulation is the end result of a complex cascade of multiple proteins and other cofactors, culminating in the formation of fibrin strands. Fibrin, formed from the precursor fibrinogen, is the protein which holds blood clots together. The coagulation cascade is described as consisting of separate pathways: the extrinsic pathway and the intrinsic pathway. (Rapaport and Rao Thromb. Haemostasis 74:7-17 (1995)). The "extrinsic" pathway is dependent upon thromboplastin, and the "intrinsic" pathway is independent of thromboplastin. The pathways converge upon the generation of thrombin, which in combination with calcium ions, converts fibrinogen to fibrin, and Factor XIII to Factor XIIIa.

A fibrin sealant exploits this final stage of the coagulation cascade and historically has been designed as a two-component system, analogous to a two-component epoxy adhesive. The first component consists of fibrinogen and Factor XIII; which can be equated to the "resin" component of an adhesive composition. Thrombin and calcium ions make up the second component, which acts ast the catalyst of the resin. The two components may be applied sequentially or simultaneously by a syringe or by spraying. When they come in contact with one another, fibrin is formed from the fibrinogen. Fibrin sealants are used for hemostasis, as well as tissue sealing in patients on heparin or with coagulation deficiencies. They promote wound healing by decreasing oozing and control air leaks by producing a fluid-tight seal at wound sites. Fibrin sealants can partially or

totally preclude the use of sutures and thereby avoid inflammatory reactions (DePalma et al., Transfusion 33:717-720 (1993)). For a detailed review of the history and use of fibrin sealant adhesive systems, see Sierra, J. Biomaterials Appl. 7:309-352 (1993).

Early surgical adhesives contained a high content of fibrinogen (about 8-10%) which could only be prepared with difficulty from fibrinogen lyophilizates. They were generally unstable and therefore required storage at -20°C to 5°C until use. Examples of these early adhesives include compositions marketed under the tradenames "Tisseel"® or "Tissucol"® (Immuno AG of Vienna, Austria), Beriplast® (Behringwerke AG, Marburg, Germany) and Biocoll® (LFB, Lille, France). These were prepared from large quantities of screened, pooled donor sourced human plasma.

Patient autologous and single-donor sourced fibrin sealants were developed in the United States in response to concerns over viral disease transmission of the commercial, pooled donor sourced fibrin sealants. These efforts were focused on producing concentrated fibrinogen, which could then be used in conjunction with readily available bovine thrombin.

In 1983, Gestring and Lerner, described a cryoprecipitation production method which utilized small amounts of patient autologous blood. (Gestring and Lerner, Vasc. Surg. 17:294-304 (1983).) This method was modified for large-scale production. (*See* U.S. Patent 4,627,879.)

Adhesive compositions incorporating other blood factor components have been described. U.S. Patent No. 4,061,731 describes a composition comprising patient autologous plasma and microcrystalline collagen and/or gelatin in combination with endogenous thrombin. These compositions and methods of use are limited by the lack of large commerically available amounts of patient blood, preparation time, which can range from an hour to overnight, and the equipment and expertise of trained hospital personnel. U.S. Patent No. 5,290,552 describes a dual-component system comprising fibrinogen, Factor XIII and collagen as one component; and thrombin and calcium ions as the other component. These two components are then mixed together just prior to use. U.S. Patent No. 4,600,574 describes a surgical adhesive comprising a flat web-like sheet of collagen, gelatin or polysaccharide which is coated with a solution of fibrinogen and Factor XIII, followed by lyophilization to form a matrix. U.S. Patent No. 4,453,939 describes a composition for the healing of wounds which comprises a web-like carrier comprised of

collagen which is coated on one side with a mixture of: (1) a fibrinogen-containing component which contains fibrinogen and Factor XIII; and (2) a thrombin-containing component. Coagulation is initiated upon insertion of the web into the patient which results in hydration and activation.

Thromboplastin (also referred to as tissue factor protein (TF)) as a therapeutic or diagnostic agent for coagulation disorders has been described. U.S. Patent No. 5,091,363 describes a composition and method for the treatment of hemophilia A. In addition, thromboplastin is used to determine the thromboplastin time (PT) of a patient as an indicator of clotting efficiency.

U.S. Patent No. 5,110,730 and PCT International Publication No. WO 94/11029 describe DNA segments defining a structural gene coding for a human tissue factor heavy chain protein and a precursor form of the protein. Methods of producing tissue factor protein recombinantly and the recombinant protein also are disclosed. The protein is disclosed to be useful for modulating the binding of Factor VII/VIIa by tissue factor *in vivo*. Diagnostic uses for detecting the presence of a thrombus or the amount of tissue factor in a body sample are disclosed.

European Patent Application Publication No. 0 278 776 discloses a tissue factor protein capable of correcting various bleeding disorders by inducing coagulation which is distinct from tissue thromboplastin because it lacks the naturally occurring lipid portion of the molecule. DNA isolates coding for tissue factor protein and derivatives thereof, recombinant expression systems for recombinant expression of the DNA are disclosed. Methods of treating coagulation disorders using the compositions also are disclosed.

European Patent Application No. 0 347 262 discloses the sequence of a cDNA coding for human tissue factor and its use for the construction of recombinant expression vectors which in transformed hosts, produce human tissue factor apoprotein, soluble human tissue factor and truncated human tissue factor for clinical and diagnostic use.

The above prior art, however, does not describe or disclose a fibrin based tissue sealant composition which utilizes thromboplastin as an initiator of fibrin formation. Indeed, the effectiveness of thromboplastin as a topical hemostatic agent has been reported to be limited when compared to conventional agents (such as thrombin). (*See* Figure 3 in PCT WO 94/02172).

## SUMMARY OF THE INVENTION

The present invention relates to tissue sealant composition that are useful to promote tissue adhesion and/or hemostasis via fibrin formation at the site of administration. In a preferred embodiment of the present invention, the tissue sealant composition is prepared as a single component containing thromboplastin and fibrinogen, which can be supplied in purified form or by adding plasma. In addition to these two essential components, the composition may further comprise calcium ions, and addition factors (also supplied in purified form or by adding plasma) such as Factors II, V, VII, X and XIII.

In another embodiment of the present invention, the tissue sealant composition is prepared in two parts (i.e. it is a "dual component system) which are mixed together before administration: one containing fibrinogen, and the other containing thromboplastin. In addition to these essential components, any or all of the aforementioned optional components can also be included in either of the two components. Preferably, mixing of the two components does not initiate fibrin formation, in which case the composition would necessarily have to be administered before complete gelling had occurred.

Using the simplest embodiment containing only thromboplastin and fibrinogen in a suitable vehicle, the tissue sealant composition reacts at the site of administration with tissues and/or blood, which supply an effective amount of calcium ions, Factors II, V, VII, X and XIII to cause the fibrinogen to be converted to fibrin. When any of these optional components are not present in sufficient quantities at the site of administration to effect fibrin formation, they are added to the tissue sealant composition prior to administration.

The present invention additionally relates to a method for promoting tissue adhesion and/or hemostasis using any of the aforementioned compositions as further described below.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a flow chart showing the blood coagulation cascade. (From Enzyme Research Laboratories, Inc., South Bend, IN.) The abbreviations for the various blood coagulation factors are included in Table I.

## MODE(S) FOR CARRYING OUT THE INVENTION

Blood coagulation is a complex cascade of events that results in formation of fibrin strands. Figure 1 illustrates the blood coagulation cascade. The various factors, their molecular weights and typical plasma concentrations are given in Table I.

**TABLE I**

| Component | Molecular Weight (daltons) | Plasma Concentration (μg/ml) | Plasma Concentration (μM) |
|---|---|---|---|
| Fibrinogen (I) | 330,000 | 3000 | 9.09 |
| Prothrombin (II) | 72,000 | 100 | 1.388 |
| Factor V | 330,000 | 10 | 0.03 |
| Factor VII | 50,000 | 0.5 | 0.01 |
| Factor VIII | 330,000 | 0.1 | 0.0003 |
| Factor IX | 56,000 | 5 | 0.08928 |
| Factor X | 58,800 | 8 | 0.13605 |
| Factor XI | 160,000 | 5 | 0.031 |
| Factor XII | 80,000 | 30 | 0.375 |
| Factor XIII | 320,000 | 10 | 0.03124 |
| Protein C | 62,000 | 4 | 0.0645 |
| Protein S | 69,000 | 10(free) | 0.1449 |
| Protein Z | 62,000 | 2.2 | 0.0355 |
| Prekallikrein | 86,000 | 50 | 0.5814 |
| HK | 110,000 | 70 | 0.6363 |
| Fibronectin | 450,000 | 300 | 0.6667 |
| Antithrombin III | 58,000 | 290 | 5 |
| Plasminogen | 90,000 | 216 | 2.4 |
| Urokinase | 53,000 | 0.1 | 0.001887 |
| Heparin Cofactor II | 66,000 | 90 | 1.3636 |
| $Alpha_2$-Antiplasmin | 63,000 | 60 | 0.9524 |
| Protein C Inhibitor | 57,000 | 4 | 0.0702 |
| $Alpha_2$-Macroglobulin | 725,000 | 2100 | 2.8966 |

An exogenous thrombin-free, fibrin based tissue sealant is described herein, which can be prepared as a single-component system. As used herein, the term "single-component" is intended to mean that the tissue sealant composition can be used alone to cause the conversion of fibrinogen to fibrin at the site of administration. In contrast,

conventional "dual-component" systems provided as two separate compositions require contacting the two compositions with one another prior to or simultaneous with administration to activate the coagulation cascade which results in *in situ* fibrin formation. The term "tissue sealant" as used herein refers to a composition which is effective to act as a tissue adhesive and/or hemostatic agent.

The use of thromboplastin as the initiator of fibrin clot formation may improve the hemostatic qualities of the adhesive by virtue of the extrinsic pathway's efficiency in forming thrombi. In this pathway, as depicted in Figure 1, thromboplastin complexes with and converts Factor VII to VIIa, and then, in the presence of calcium ions, converts Factor X into Factor Xa, and Factor V into Va creating a "prothrombinase complex." In turn, Factor Xa (which uses Factor Va as a cofactor) converts Factor II (prothrombin) into thrombin. Thrombin then converts fibrinogen into fibrin which forms a clot, and also converts Factor XIII into Factor XIIIa in the presence of calcium ions. Factor XIIIa in turn causes covalent crosslinking of the fibrin clot which makes it more stable both mechanically and proteolytically.

Accordingly, the tissue sealant composition contains, as its primary elements, an effective amount of thromboplastin and fibrinogen. Additionally, the tissue sealant composition preferably contains calcium ions. In the case of a tissue sealant composition containing only thromboplastin, fibrinogen and calcium ions in a suitable delivery vehicle, all other factors (and other requirements) which are necessary to effect fibrin formation are found at the application site. However, not all application sites, especially those which are not actively bleeding, provide a sufficient source of these additional components. In this situation, the tissue sealant preferably contains all of the necessary factors required for fibrin formation, i.e. thromboplastin, fibrinogen, calcium ions and Factors II, V, VII, X and XIII.

In a preferred embodiment, the tissue sealant compositions are prepared as a single-component system. There are advantages of the single-component system over conventional two-component fibrin sealant systems now in place. No exogenous thrombin

of either human or other source is required, especially in large quantities. This eliminates concerns over immunologically induced coagulopathies from bovine sourced thrombin preparations. The need for large quantities of exogenous thrombin are eliminated in that very small amounts of thromboplastin enable the conversion of prothrombin to thrombin. Another important advantage of the composition and its use is its that it eliminates the need for catalysis of the "resin", which facilitates uniform mixing, curing and subsequent strength of the material. The convenience is improved in that only one delivery device or dosage unit is required to prepare and apply the material.

Sources of Composition Components

Thromboplastin can be obtained from brain or tissue extracts, or it can be prepared using recombinant techniques. Thromboplastin which is obtained from natural sources contains an amount of lipid associated therewith which is necessary for thromboplastin activity. Thromboplastin which is produced from recombinant techniques must be "lipidated" to restore its native activity. As used herein, the term "thromboplastin" refers to thromboplastin from natural sources, as well as lipidated thromboplastin produced using recombinant techniques. See, for example, U.S. Patent No. 5,314,695 which describes the lipidation procedure.

Recombinant thromboplastin is commercially available from Ortho Diagnostics (Raritan, N.J.) sold under the tradename RecombinPlastin® and Baxter Healthcare Corporation, Dade Division provides a thromboplastin extract sold under the tradename Innovin® (Miami, Fl.) Methods of preparing purified thromboplastin are well known in the art and are described, for example in U.S. Patent Nos.: 5,254,350; 4,755,461; 5,270,451; 3,522,148; 3,522,148 and European Patent Publication No. 524 803 A2.

Purified constituents are commercially available or readily obtainable from human and animal blood fractions or can be recombinantly produced using methods well known to those of skill in the art. It should be appreciated that the constituents as noted herein can be obtained from any suitable animal source, e.g., human, bovine or porcine. For example,

bovine fibrinogen is commercially available from Sigma Chemical Co. (Saint Louis, MO); Factors V, VII and XIII are commercially available from American Diagnostics Inc. (Greenwich, CT); Factor IX (human and bovine) is commercially available from Accurate Chemical & Scientific Corp. (Westbury, N.Y.) and American Diagnostic Inc. (Greenwich, CT); human and rabbit Factor VIII is commercially available from Accurate Chemical & Scientific Corp. (Westbury, N.Y); and human Factor X can be purchased from American Diagnostics Inc (Greenwich, CT) or bovine Factor X can be purchased from Sigma Chemical Co. (St. Louis, MO).

Human or animal plasma can be used "as is" as a source for the various constituents after removal of the cellular components of blood by centrifugation. For example, plasma can be processed to prepare a plasma cryoprecipitate by freezing, thawing and further centrifugation, which can be used as a source of fibrinogen and Factor XIII. Various factors can also be isolated from plasma which is in crystalline or amorphous form, or as a lyophilizate. Also, Factors II, V, VII and X can be obtained from a cryosupernatant of plasma.

Fibrinogen and Factor XIII can also be obtained from allogeneic or autologous plasma preparations.

Fibrinogen and Factor XIII may be obtained from the "resin" component of commercially available dual-component fibrin sealant compositions. For example, bovine fibrinogen can be obtained from a fibrin sealant preparation such as Tisseel® (Immuno AG, Vienna, Austria.)

In addition, Factors II, V, VII and X can be obtained from anti-hemophilia B therapeutic agents (Octapharma, Immuno AG, Alpha Therapeutics, Baxter-Hyland and Armour Bayer).

Factor II may be produced by recombinant expression techniques as described in U.S. Patent No. 5,476,777. In addition, purification methods for Factor II are described in Miletich et al. Meth. Enzymol. 80:221-228 (1981) and U.S. Patent No. 5,378,365.

Factor VIII can also be produced by the method disclosed in European Patent No. 085,923, incorporated herein by reference. Additional methods of preparing and isolating recombinant Factor VIII as well as purifying Factor VIII are well known to those of skill in the art as evidenced by Wood et al. Nature 312:330-336 (1984); U.S. Patent No. 5,422,260; U.S. Patent No. 5,422,250; U.S. Patent No. 5,410,022; and U.S. Patent No. 5,738,612.

Methods of producing, isolating and purifying Factor IX are well known to those of skill in the art as evidenced by U.S. Patent No. 5,286,849; U.S. Patent No. 5,171,569; and Kaufman J. Biol. Chem. 261:9622-9628 (1986).

Factor X can be produced as described in Miletich et al. supra.

Preparation of the Tissue Sealant Compositions

To produce the tissue sealant, the desired constituents are initially produced in soluble form and, where appropriate, are virally deactivated. As is known to those of skill in the art, when the components are purified from a native or natural source, they are provided in purified or substantially purified form. "Purified" shall mean that the protein or factor of interest is substantially free of cellular and other biological components normally associated with the protein or factor in its native or natural environment in the cell or body fluid. Thus, the term "purified" can be used to describe proteins and factors isolated from their native environment or isolated from a biological, non-naturally occurring environment such as when they are recombinantly produced in a host cell such as a Chinese Hamster Ovary cell which is commercially available from the American Type Culture Collection ("ATCC"; Rockville, MD).

It should be understood, although not always explicitly stated, that the compositions of this invention can include, in addition to the factors in forms as they appear in nature, i.e., in a "purified" state, analogs, muteins, conjugates, and homologues of the proteins or factors, provided that the biological activity of the factor is not substantially impaired. "Substantially impaired" would include a greater than 50%

reduction in the biological activity of the analog, homologue or mutein, as compared to native or natural protein or factor. Accordingly, use of a term such as "thromboplastin", in addition to thromboplastin from natural sources, is intended to encompass all alternative forms of thromboplastin having a biological activity which is not substantially impaired, i.e. "thromboplastin equivalents." The biological activity of a protein or factor includes any feature of the polypeptide determined by suitable experimental investigation, including, but not limited to the experiments set forth herein relating to coagulation time and the ability to promote the formation of fibrin *in situ*.

The preferred single-component composition which contains thromboplastin, fibrinogen and all of the necessary extrinsic pathway factors (Factors II, V, VII, X and XIII) is prepared by mixing the constituents together either without calcium ions or an amount of calcium ions which is insufficient to effect gelation (≤3mM) within an hour. Such compositions remain flowable for at least one hour, or until they come in contact with tissue and/or blood after administration. The additional $Ca^{2+}$ from blood or tissue permits the extrinsic pathway reactions to occur, which results in fibrin clot formation.

Alternative embodiments of the tissue sealant compositions of the present invention are described below in Table II:

**TABLE II**

| Single/Dual Component | First Component* | Second Optional Component | Missing Component(s) ** | Reaction Started by: |
|---|---|---|---|---|
| Single | Fibrinogen <3mM $Ca^{2+}$ Factor II Factor V Factor VII Factor X Factor XIII | None | Additional $Ca^{2+}$ (sufficient to bring total concentration to > 5mM) | Administration to site which provides additional $Ca^{2+}$ |
| Single | Fibrinogen <3mM $Ca^{2+}$ | None | Factor II Factor V Factor VII Factor X Factor XIII Additional $Ca^{2}$ | Administration to site which provides all missing components |
| Dual | <3 mM $Ca^{2+}$ | Fibrinogen Factor IX Factor XIII | Factor II Factor V Factor VII Factor X Additional $Ca^{2+}$ | Adding Plasma containing missing components*** |
| Single | Factor XIII Fibrinogen >5mM $Ca^{2+}$ | None | Factor II Factor V Factor VII Factor X | Administration to site which provides all missing components |
| Dual | > 5 mM $Ca^{2+}$ | Fibrinogen Factor XIII Factor II | Factor V Factor II Factor X | Administration to site which provides all missing components |

* In addition to thromboplastin.

** The missing component(s) is what is found at the application site that triggers the extrinsic pathway reactions to occur, resulting in conversion of fibrinogen to fibrin.

*** Must be administered immediately after adding plasma.

"An effective amount" of the individual components is an amount that, when combined as described herein and brought into contact with body tissues *in situ*, will induce the conversion of fibrinogen to fibrin which results in fibrin clot formation. Suitable concentrations for most of the factors correspond to a range present in normal human plasma and as provided in Table III, below. It should be assumed, although not always explicitely stated, that "effective amounts" of the components are used and incorporated into the compositions of this invention.

**TABLE III**

| Component | Effective Concentration Range (mg/mL) | Preferred Concentration Range (mg/mL) |
|---|---|---|
| Fibrinogen | 1-200 | 2-120 |
| Factor II (prothrombin) | 0.001-1.0 | 0.10-0.50 |
| Factor V | 0.0001-0.03 | 0.001-0.02 |
| Factor X | 0.001-0.08 | 0.03-0.07 |
| Factor XIII | 0.0001-0.04 | 0.001-0.05 |
| Factor VII | 0.00001-0.004 | 0.0001-0.003 |
| thromboplastin | 0.00005-5 | 0.0001-.001 |
| Calcium ion (Ca(II)) | 0.5-30 mM | 2-20 mM |

Optional Components

This invention also provides compositions comprising a single tissue adhesive in combination with other constituents, such as stabilizers, preservatives, therapeutics collagen, collagen analogs and collagen conjugates. Any stabilizer that functions to maintain the activity of the tissue adhesive upon administration to the patient can be used in practicing the invention. Examples of such stabilizers include, but are not limited to

Tris (trishydroxymethylaminomethane), PIPES (Piperazine-N,N-bis(2-ethane-sulfonic acid, 1.5 sodium salt), imidazole, and MOPS (3-(N-Morpholine) propanesulfonic acid). Suitable preservatives include sodium azide, thimerosal, BHA, BHT. Other preservatives that function to prevent the growth of microorganisms that would damage the component system is suitably added to the adhesive components.

Collagen, a collagen analog or a collagen-containing conjugate can be added to increase the rate of gelation, and also to thicken the adhesive composition and augment cohesiveness. The amount of collagen to be added can be easily determined by varying the amount of collagen and chosing that concentration of collagen which gives the desired result. The collagen may be atelopeptide collagen or telopeptide collagen. Animal or human-based collagen is suitably used and can be purified using methods well known to those of skill in the art and described in U.S. Patent No. 4,233,360. These collagen preparations also are available commercially from a supplier such as Collagen Corp. (Palo Alto, CA) under the tradename Zyderm II®. Other biomaterials may be used to augment either the physical performance of the sealant or its application in a specific repair site. For example, hydroxylapatite or tri-calcium phosphate can be incorporated for repairs in bony tissue. Attachment factors such as RGD peptide sequences can be added as well. Additional biomaterials include, but are not limited to bone or hard tissue materials, plastics, particulates and metals. As used herein, an analog is intended to include the materials as described above having similar and different chemical or physical entities of the same material as naturally occurring in nature or purified from a native source. An analog can consist of hybridized or conjugated proteins, as described in published PCT International Publication No. WO 94/16085.

Therapeutic agents can also be added, in which case the tissue sealant additionally serves as a vehicle for delivery of these components. For example, components such as antibiotics, metabolic substances, cells and growth factors can be added. Growth factors such as EGF, TGF-α, TGF-ß, FGF, PDGF can be added. Cytokines such as interleukin or stem cell factor also can be suitably added. Antibiotics can be added and are particularly

useful when the adhesive is applied to exposed wound sites such as mouth sores and burns. The tissue sealant compositions can also be mixed with cells, autologous, cultured or modified, allogeneic or xenogeneic. As is apparent to those skilled in the art, the amount of an added component will vary with the use of the adhesive and the recipient but is easily determined by the treating physician.

Factors VIII and IX are optionally added to overcome tissue factor pathway inhibitor (TFPI) which shuts down Factor X activation by the VIIa/thromboplastin complex that may occur in a physiologically complex mixture. However, this interaction is preferably minimized in the tissue sealant compositions of the present invention by using sufficiently purified materials.

Factor VIII and IX can be obtained from plasma as described above for the other factors using known methods. In addition, Factor VIII can be obtained from anti-hemophilia A therapeutic agents (Octapharma, Immuno AG, Alpha Therapeutics, Baxter-Hyland and Armour Bayer).

A calcium ion chelator can also be incorporated in an amount effective to prevent formation of a fibrin clot prior to administration while still allowing the formation of clot upon administration. The amount of chelator will vary based on the source of the reagents and ultimate use of the composition. Any calcium ion chelator that functions similarly to ethylenediaminetetraacetic acid (EDTA) in binding/chelating ions or other metal ions can be used in the practice of this invention. Examples of suitable chelators include, but are not limited to citrate, salts of citrate, ethylene-bis (oxyethylenenitolo) tetraacetic acid (EGTA) and salts of EGTA.

Administration and Use

The tissue sealants of the present invention are applied to human (or animal) tissue which provides physiological calcium from the surrounding tissue (and optionally additional necessary components), resulting in fibrin formation.

The tissue sealants of this invention can be used in a wide variety of procedures and surgical indications, adjunctively as a replacement for sutures, hemostatic agents, packing

materials and to deliver various therapeutic agents. The tissue sealants can be used in any application where the formation of a fibrin clot is desired, hemostasis is required or where prior art surgical and fibrin sealants were previously used.

Historically, fibrin sealants have been used in tissue remodeling and wound repair. They also have been shown to act as osteogenic or osteostimulatory agents. Cardiovascular applications are numerous. For example, fibrin sealants have been used as a hemostatic sealant for vascular graft attachment, cardiovascular patches, heart valve attachment and to preclot vascular grafts.

Fibrin sealants are also used to deliver drugs and antibiotics. Grafting of skin with fibrin sealants has been successful for burn patients, face lifts and in rhinophyma repair.

Accordingly, the compositions described herein can be used to initiate the formation of fibrin or a fibrin clot *in situ*. Further provided are methods of treating bleeding disorders and treating wounds in a patient. These methods require administering to the patient a therapeutically effective amount of the single-component adhesive composition as described herein to form fibrin.

As used herein, "administering" shall mean providing the recipient or patient with the tissue sealant topically for local therapy or administered by injection intravascularly (if the sealant is to be used as an embolic agent) by combining the adhesive with a suitable pharmaceutically acceptable carrier such as phosphate buffered saline. Other suitable pharmaceutical carriers, stabilizers and preservatives are well known to those of skill in the art and are described for example in Remington's Pharm. Sci. 15th Ed. (Mack Publ. Co., Easton (1975)).

Local administration of the adhesive can be in a single dose or multiple doses as determined by the treating physician. In any case, the dosage must contain an effective amount of thromboplastin and fibrinogen, as well as any other optional components, such that administration causes fibrin formation.

As is apparent to those of skill in the art to which this invention pertains, the compositions of this invention can be combined with standard carriers and preservatives to

form pharmaceutical compositions, which are also within the scope of this invention. Accordingly, the use of the compositions described herein to prepare medicaments for promoting and/or inducing the formation of fibrin *in situ*, is further within the scope of this invention. This invention furhter provides uses of the above composition for the preparation of medicaments for inducing the formation of fibrin *in situ* in an animal such as a rat, guinea pig, rabbit or a human patient.

As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. It could, however, exclude other, non-specified blood coagulation factors that can impair or substantially alter the ability of the formulation to remain flowable prior to administration and/or can substantially alter or impair the ability to promote the formation of fibrin *in sit.*, e.g., TFPI, thrombin, and fibrin catalysts. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

THE FOLLOWING EXAMPLES ARE OFFERED BY WAY OF ILLUSTRATION AND NOT BY WAY OF LIMITATION.

## EXAMPLE I

### Effects of Varying Composition Components and Concentrations

This example shows the effect of adding calcium ions and/or collagen to the tissue sealant composition. It also shows that the formulations are stable at room temperature and are effective, *in vivo*, as well as the impact of increasing the fibrinogen concentration on gelation time. In general, the compositions were prepared as follows: bovine source citrated plasma which served as a source of fibrinogen and Factor XIII, was recycled three times (cryoprecipitate) as prepared by a modification of the method of (Gestring and Lerner Vasc. Surg. 17:294-304 (1983)) and was mixed 1:1 (v/v) with reconstituted human reference plasma which served as a source of Factor II, V, VII, X, and as an additional source of fibrinogen and Factor XIII (Sigma Chemical Co., Product #A7432). The plasma mixture thus formed was then mixed with the following additional componenents to achieve the concentrations given below in Table III: thromboplastin (TP) (RecombiPlastin (lipidated) from Ortho Diagnostics) having a nominal thromboplastin concentration of 200 ng/ml; and Zyderm II collagen (65 mg/ml)(Collagen Corp., Palo Alto, CA). The components were mixed at room temperature in a culture tube by vortexing. The time for the mixture to form a gel was observed and recorded after the addition of 0.2 mL of a 40 mM $CaCl_2$ solution and the results are shown in Table IV.

TABLE IV

| Plasma (mL) | Cryoppt. (mL) | Collagen (mL) | TP in TBS (mL) | $[Ca^{2+}]$ (mM) | Gel Times (seconds) |
|---|---|---|---|---|---|
| 0.5 | 0 | 0 | 1.0 | 0 | no gel |
| 0.5 | 0 | 0 | 1.0 | 5 | 30 |
| 0.25 | 0.25 | 0 | 1.0 | 5 | 20 |
| 0.25 | 0.25 | 0.5 | 1.0 | 5 | 15 |

These results show that the reference plasma did not gel upon the addition of TP alone. When the calcium ion concentration was raised to 5 mM, gelation occurred at 30 seconds. The addition of more fibrinogen and Factor XIII as well as collagen increased the gelation rate of the sealant.

## EXAMPLE II

*In Vitro* Stability of Differen Compositions

To assess the *in vitro* stability of preparations containing thromboplastin, the protocol as outlined above was followed, except formulations were allowed to incubate for 1 hour with $Ca^{2+}$ at 2.0 mM, then 0.2 mL of 40 mM $CaCl_2$ solution were added (net $[Ca^{2+}]$= 4.6 mM), and the results are shown in Table V.

TABLE V

| Plasma (mL) | Cryoppt (mL) | Collagen (mL) | TP in TBS (mL) | Gel Time (seconds) |
|---|---|---|---|---|
| 0.5 | 0 | 0 | 1.0 | 25 |
| 0.25 | 0.25 | 0 | 1.0 | 25 |
| 0.25 | 0.25 | 0.5 | 1.0 | 15 |

These results show that gelation times decreased from 25 to 15 seconds in the presence of collagen, and that the material did not gel prematurely at a low $Ca^{2+}$ concentration in less than one hour.

## EXAMPLE II

### Efficacy of the Tissue Sealant as a Hemostatic Agent

The suitability of the single-component tissue sealant as a hemostatic agent was evaluated in a standardized rodent liver incision model and the results are shown in Table VI. A midline incision transecting the abdomen was made in an anesthetized rat. The liver was elevated and exposed. A lobe was completely bisected anterior to posterior. Once hemorrhaging was established, the sealant was applied along the incision and the time for visible bleeding to stop was recorded. Fibrin sealant and a fibrin-collagen composite tissue adhesive containing thrombin was also tested.

**TABLE VI**

| Composition | Time to Hemostasis (seconds) |
|---|---|
| Prior Art Fibrin Sealant (fibrinogen, 60 mg/mL; thrombin, 200 U/mL; and $CaCl_2$, 40 mM) | 15 |
| Prior Art Two Component Tissue Adhesive (thrombin, 200 U/mL, fibrinogen, 30 mg/mL; collagen 20 mg/mL, and $CaCl_2$, 40 mM. See U.S. Pat. No. 5,290,552) | 10 |
| Single Component Sealant (as describd in the first example given in Table V) | 12 |

As shown, the single component sealant is nearly as effective as the two component tissue adhesive, and is more effective than the prior art fibrin sealant.

Throughout this application, various disclosures, including published patent applications, issued patents, journal articles and textbooks, are referenced. The disclosures of these materials are incorporated by reference into this application, as if each reference

were individually indicated to be incorporated by reference, to more fully describe the state of the art to which this invention pertains.

As is apparent to one of ordinary skill in the art, many changes and modifications can be made to the above embodiments without departing from the spirit and scope of the following claims.

What is claimed is:

1. A single-component tissue sealant composition comprising thromboplastin and fibrinogen.

2. The composition according to claim 1 further comprising $Ca^{2+}$ ions at a concentration less than or equal to 3.0 mM.

3. The composition according to claim 2 further comprising collagen.

4. The composition according to claim 1 further comprising Factors II, V, VII, X and XIII.

5. The composition according to claim 1 further comprising plasma, wherein said plasma comprises Factors II, V, VII, X and XIII.

6. A dual-component tissue sealant composition comprising an admixture of:

(a) a first component comprising an effective amount of fibrinogen; and

(b) a second component comprising an effective amount of thromboplastin.

7. The dual-component tissue sealant composition according to claim 6 wherein:

(a) said first component further comprises $Ca^{2+}$ in an amount greater than or equal to 5 mM; and

(b) said second component further comprises Factors II, V, VII, X and XIII.

8. A method for promoting tissue adhesion and/or hemostasis at a local tissue site in an animal comprising administering a single-component tissue sealant composition to said site, wherein said tissue sealant composition comprises thromboplastin and fibrinogen.

9. The method according to claim 8, wherein said tissue sealant composition further comprises $Ca^{2+}$ ions in a concentration less than or equal to 3 mM.

10. The method according to claim 9, wherein said tissue sealant composition further comprises collagen.

11. The method according to claim 8, wherein said tissue sealant composition further comprises Factors II, V, VII, X and XIII.

12. The method according to claim 8, wherein said tissue sealant composition further comprises plasma, wherein said plasma comprises Factors II, V, VII, X and XIII.

13. A method for promoting tissue adhesion and/or hemostasis at a local tissue site in an animal comprising administering a dual-component tissue sealant composition to said site, wherein said dual-component tissue sealant composition comprises an admixture of:

(a) a first component comprising fibrinogen; and

(b) a second component comprising thromboplastin.

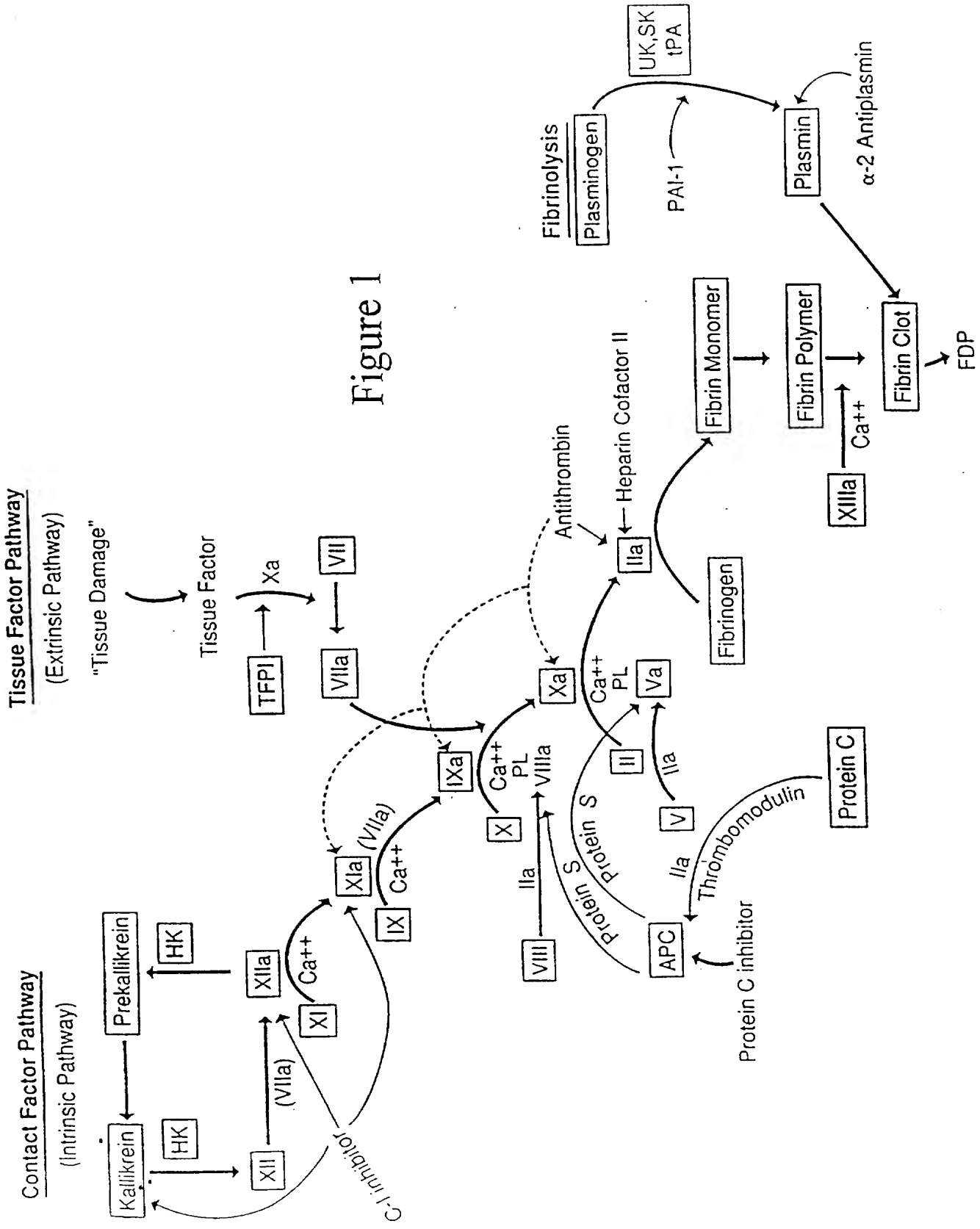
Tissue Factor Pathway
(Extrinsic Pathway)
"Tissue Damage"
Tissue Factor
TFPI
Xa
VII
VIIa
Contact Factor Pathway
(Intrinsic Pathway)
Kallikrein
HK
XII
(VIIa)
XIIa
Prekallikrein
HK
XI
Ca++
XIa
(VIIa)
IX
Ca++
IXa
X
Ca++
PL
VIIIa
Xa
VIII
IIa
Antithrombin
Heparin Cofactor II
IIa
Ca++
PL
II
Va
V
IIa
Protein S
Protein S
APC
IIa
Thrombomodulin
Protein C
Protein C inhibitor
C-1 Inhibitor
Fibrinolysis
Plasminogen
UK,SK
tPA
PAI-1
Plasmin
α-2 Antiplasmin
Fibrin Monomer
Fibrinogen
Fibrin Polymer
XIIIa
Ca++
Fibrin Clot
FDP

Figure 1

# INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/US 97/02614

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | DATABASE BIOSIS<br>BIOSCIENCES INFORMATION SERVICE,<br>PHILADELPHIA, PA, US<br>SUZUKI M ET AL: "CLINICAL APPLICATION OF<br>THE FIBRIN ADHESIVE."<br>XP002033879 | 1,6,8,13 |
| Y | see abstract<br>& OTOLARYNGOLOGY (TOKYO) 56 (11). 1984.<br>949-953. CODEN: JIBKAM,<br>--- | 1,2,8,9 |
| Y | US 5 407 671 A (HEIMBURGER NORBERT ET AL)<br>18 April 1995<br>see column 3, line 42 - column 3, line 54;<br>claim 1; example 1<br>---<br>-/-- | 1,2,8,9 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 26 June 1997 | 08.07.1997 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. ( + 31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: ( + 31-70) 340-3016 | Heck, G |

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# INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 97/02614

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | PATENT ABSTRACTS OF JAPAN<br>vol. 014, no. 431 (C-0759), 17 September 1990<br>& JP 02 167234 A (FURUKAWA FUAAMASHII:KK;OTHERS: 02), 27 June 1990,<br>see abstract | 1,2,4,8, 9,11 |
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